Plantlet Production of *Swietenia macrophylla* King through Tissue Culture

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Abstract

Different vegetative parts of *Swietenia macrophylla* King were used as explants in *in-vitro* studies. They were cultured in MS medium supplemented with various plant growth regulators. Adventitious shoots could be obtained from the friable callus when the seedling nodal segments were cultured on BA (2, 5 ppm) media. The regenerated shoots could be rooted to form whole plantlets which could be transferred to soil.

Introduction

There has been considerable progress made recently in regenerating plantlets of tropical hardwood trees through tissue culture (Lee & Rao, 1981; 1986; 1987, Rao & Lee, 1986, Rao, 1988). The technique has great potential for exploitation in the mass production of propagules of forest tree species to implement afforestation and reforestation programmes. In this paper, the *in-vitro* culture response of various explants of *Swietenia macrophylla* King (Broadleaf Mahogany) is reported. The usefulness of *S. macrophylla* is very clearly explained in the tree flora of the S.E. Asian region (Corner, 1952).

Materials and Methods

Seedlings of *S. macrophylla* were established in sand. Both nodal and internodal segments of about 0.5 cm length were used as explants. Nodal segments from young basal shoots of some 10-year old trees were also used in the experiments. These explants were lightly washed in running water and later surface sterilised for about 10 minutes in 10% (w/v) freshly prepared sodium hypochlorite solution. Tween 20 was added as wetting agent. To reduce the exudation of phenolic compounds into the media, the nodal segments from the trees were soaked in sterile water for about 2 hours, after surface sterilisation. Seedling nodal segments did not require this treatment. Leaf tissue from shoots regenerated under *in-vitro* conditions were also used to test their growth response.

Murashige & Skoog (1962) medium was used with 2% sucrose and 0.7% Difco Bacto Agar. The macronutrients were reduced to half strength to lower the salt concentration. However, the concentration of ammonium nitrate was increased to 2 g/l to increase the level of reduced nitrogen. The media were supplemented with different concentrations and combinations of benzyladenine (BA), Indole-acetic acid (IAA), Indole-butyric acid (IBA), and Kinetin (K). All cultures were maintained at

22°C to 25°C with a photoperiod length of 16 hours of Philips white flourescent light at 35–45 μmole. $em^{-2}s^{-1}$, followed by 8 hours darkness. Each treatment was replicated 10 times and each experiment was repeated twice. For histological studies, selected tissues of varying ages developed *in-vitro* were fixed in formalin acetic alcohol (1:1:18). Standard practices were followed for dehydration, microtoming and staining with hematoxylin and erythrosin (Sass, 1968).

Results

Response of explants on IAA + K media on a 5^2 factorial combination: Both seedling explants as well as those excised from the mature (10–year old) trees were inoculated onto IAA and K media (0, 1, 2, 5 and 10 ppm each), in a 5^2 factorial combination. The nodal segments of young shoots excised from 10–year old trees remained green without much response. Although the axillary buds in some replicates (IAA [0 to 2 ppm] and high K [5 to 10 ppm]) enlarged to some extent, they remained dormant and no shoots developed. About 60% of the replicates turned brown about 12 days after inoculation and there was no further growth.

Growth was also slow in the nodal and internodal segments from the seedling axis. All segments remained fresh. The explants on media with high IAA (5, 10 ppm) and K (0 to 10 ppm) started growing 25 to 30 days after inoculation. In about 70% of the cultures, the middle region of the segments enlarged and small 'swellings' developed when they were subcultured onto fresh medium 40 days after the first inoculation. In cultures of about 50 to 60 days old, many small lobes of compact callus developed which enlarged further in 60 to 70 days (Fig. 1). In all the cultures, the axillary buds remained dormant, and did not emerge as shoots.

Response of nodal segments on BA media: In view of the limited response seen above, an attempt was made to induce multiple shoots by culturing nodal segments both from seedlings and old trees, in BA (0 to 10 ppm) media. The nodal segments from the trees failed to respond. About 50% of the replicates turned brown within 10 to 12 days after inoculation and subsequently died.

With the nodal segments from seedlings, growth improved in all the media used. In BA media (0.1 and 1 ppm), the axillary buds sprouted into shoots about 20 days after inoculation. Shoot growth was slow and within 14 days from sprouting, the tiny leaves that developed defoliated. No subsequent development was observed, irrespective of the concentration used.

Figure 1 to 8. *(opposite page)*

Fig. 1. Compact callus developing from a nodal segment cultured on IAA (5.0 ppm) + K (2.5 ppm) medium (60 days). Many lobes were obtained but growth is limited.

Fig. 2. Small shoots with whitish leaves developing from the callus cultured on BA (2 ppm) medium (70 days).

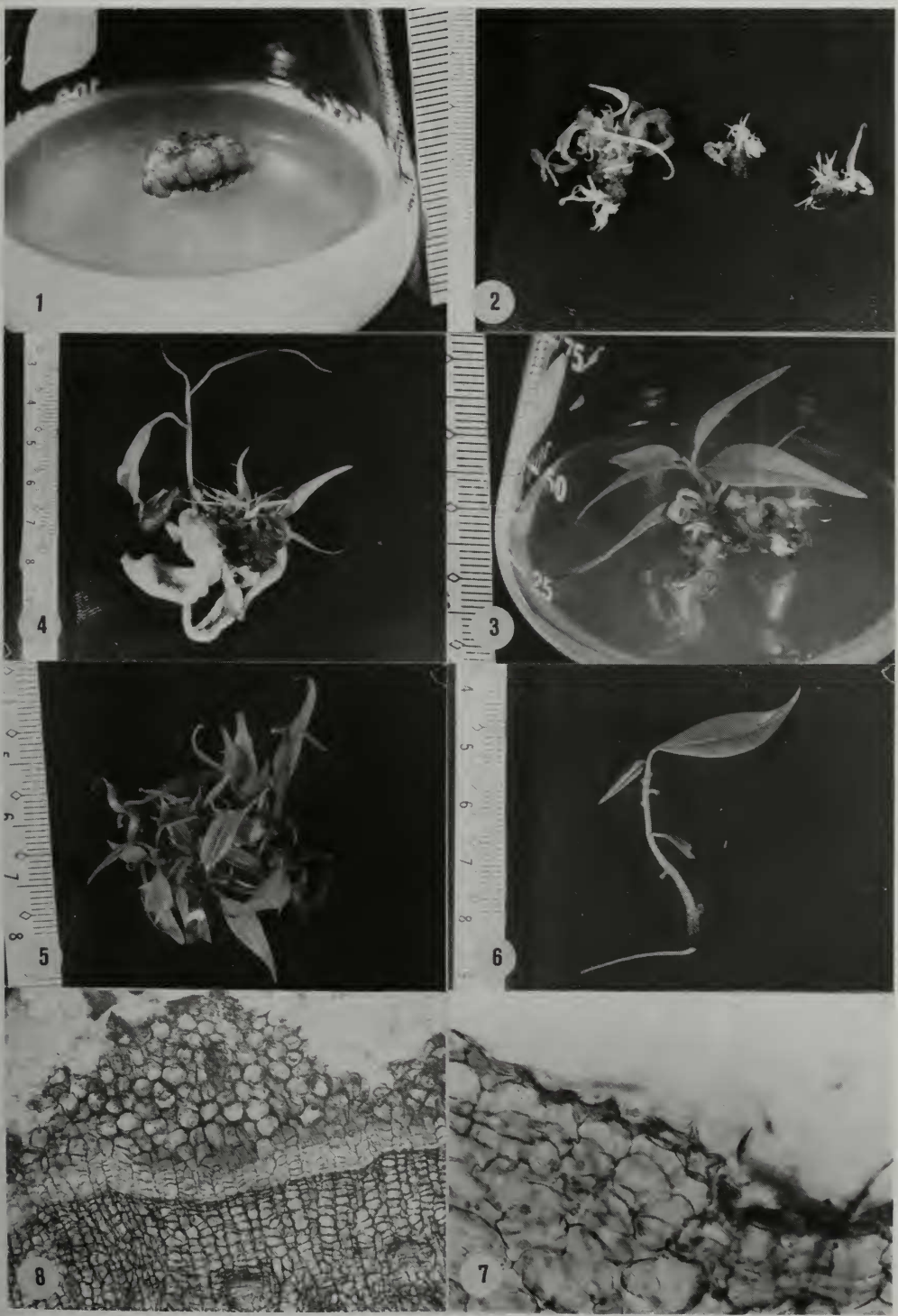
Fig. 3, 4, 5. Several shoots obtained from the callus subcultured continuously on BA (2 ppm) medium. These were the 4th subculture and they were about 120 days after inoculation.

Fig. 6. A rooted plantlet of *S. macrophylla,* with a single root.

Fig. 7. A periderm was formed at the cut surface of the nodal segment that was immersed in BA (2 ppm) medium (10–12 days). Note the cambial initiation.

Fig. 8. About 6 to 8 layers of cambial tissue were formed (15 to 20 days). The cells on the outer region were rounded and large.

Figure 1 to 8



The best response was obtained in BA (2 and 5 ppm) media. The axillary buds sprouted 10 days after inoculation, with each bud giving rise to a small reddish brown shoot. The growth was vigorous and shoots measured 1.5 cm in length in about 25 to 30 days. Thereafter, the growth rate slowed down, and the lower leaves defoliated. At this stage, the shoot segments were subcultured onto fresh media of similar composition, otherwise, the new axillary shoots would remain dormant and leafless. Even after subculturing, only slight elongation of the shoots was observed and no multiple shoots developed.

Along with the axillary shoots, callus developed at the basal region of the segments in about 2 weeks (Fig. 2). The growth progressed further and about one month after inoculation, the surface layers ruptured lightly at certain loci, to release small specks of whitish and friable callus. These were subcultured once in 3 weeks to encourage further tissue growth.

About 9 weeks after the first inoculation, small whitish buds developed on the surface of the brown callus. Some of these developed into shoots with whitish or pale coloured leaves (Figs. 2, 3), and in 10 days after emergence, the leaves turned greenish. These shoots grew to a height of 1 to 3 cm within 25 days after emergence. A cluster of 6–7 shoots was formed around a single clump of friable callus (Figs. 4, 5), and these shoots were excised for rooting. After excision of the mature shoots, new shoots were induced when the brownish and friable callus clump was subcultured onto a fresh medium.

Anatomical studies showed that about 10 to 12 days after inoculation, a distinct periderm was formed at the cut surface of the nodal segment and a regular cambial zone was initiated below the damaged peripheral layers (Fig. 7). There was no visible change observed in the original cells in terms of losing their contents before the regeneration of the new cambium. The fully formed cambial zone was 5 to 6 layers deep and prominent (Fig. 8). The cambial formation was distinct but the distribution was discontinuous (Fig. 9). The new cells formed towards the exterior from the cambial activity were big, loosely arranged and of various sizes and shapes. Most of the peripheral cells were either rounded or elongated. In 2 to 3 weeks old callus,

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Figure 9 to 16. *(opposite page)*

Fig. 9. There were variations in cambial distribution and formation of loosely arranged tissue. The peripheral cells were of various shapes and sizes (25 days).

Fig. 10. A mass of compact callus tissue which contained meristematic layers divided in many planes, contributing to the growth of the callus mass. The peripheral cells were loosely arranged.

Fig. 11. Many growth nodules were present in some callus clumps. These consisted of meristematic cells which divided actively, contributing to the growth of the callus.

Fig. 12. Mature callus (60 days) with loosely arranged tissue, with a shoot primordium.

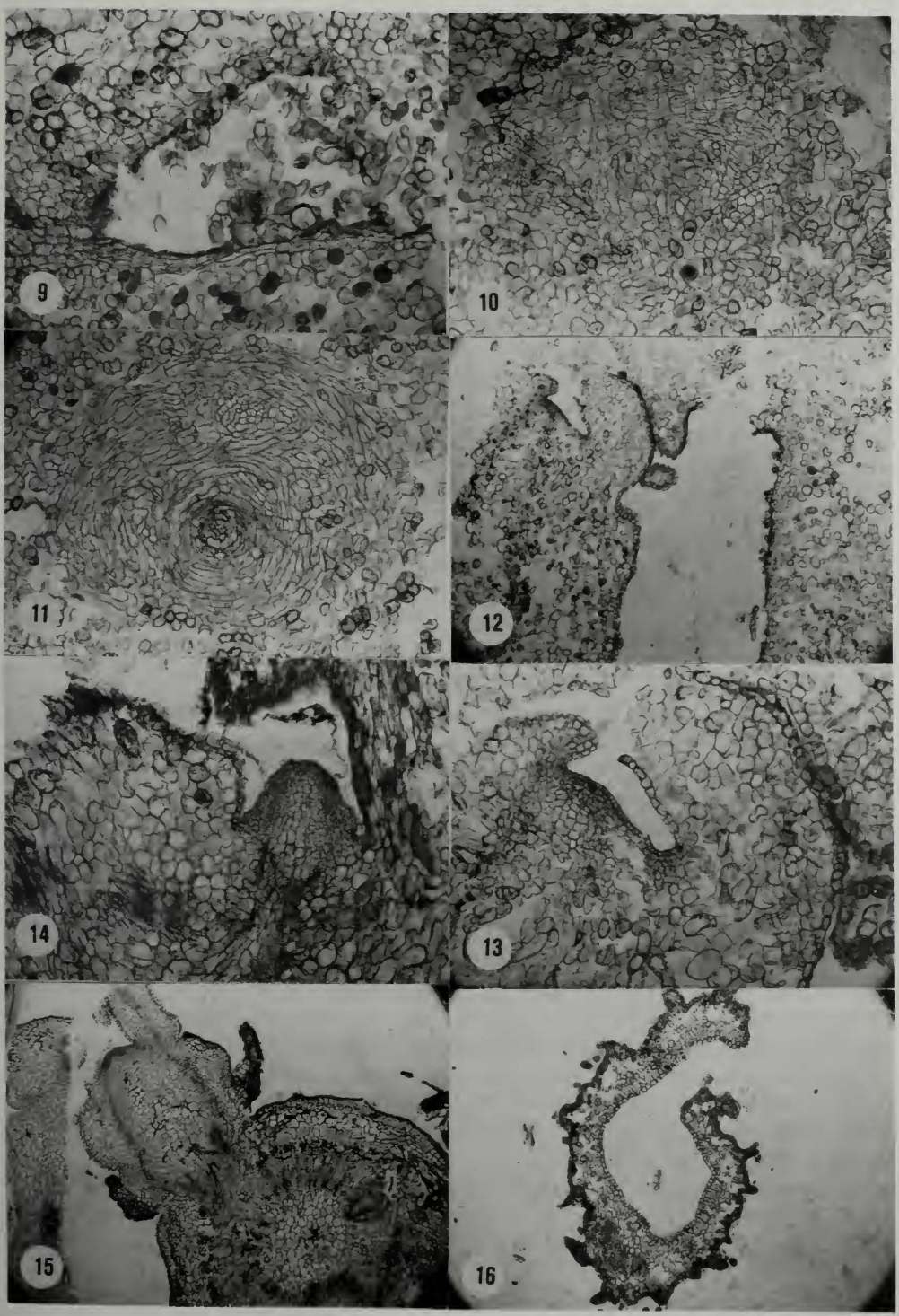
Fig. 13. The shoot primordium from Fig. 12, enlarged to show the shoot apex. The tunica-corpus zones were distinct.

Fig. 14. An axillary bud of a regenerated shoot, induced to grow in a BA (2 ppm) medium.

Fig. 15. New adventitious shoot growing directly from the secondary stem axis, in BA (2 ppm) medium (95–100 days).

Fig. 16. Leaf cultured on BA (2 ppm) medium (85 days). Several outgrowths from the lower epidermal layers were observed. Some of the outgrowths consisted of 8 to 10 cells (glandular) and could be detached from the epidermal layers.

Figure 9 to 16



the inner tissue was also compactly arranged although the peripheral free cells were loosely arranged (Figs. 9, 10). Within the new tissue, there were many meristematic regions and through their activity, additional tissue was formed, thereby contributing to the growth of the callus (Figs. 10, 11).

In callus 5 to 6 weeks old, distinct 'growth nodules' were present. Cells within the 'growth nodules' were compact and radially arranged in concentric rings (Fig. 11). These cells as well as those surrounding them were highly meristematic and through their active division, the volume of the callus increased. The cells towards the peripheral region of the 'growth nodules' were more rounded and they were loosely arranged, some with tannin contents (Fig. 11). The old tracheal cells remained in the centre with the new cell layers around them (Fig. 11). In the newly formed tissue 2 islands of secondary phloem were regenerated.

Shoot primordia developed from the friable callus in about 60 days after inoculation (Fig. 12). In the axis the cells of different sizes and shapes were loosely arranged with many air spaces bounded by a distinct epidermis. The peripheral layers remained fairly intact despite loose tissue inside. At certain loci, a single or a group of meristematic cells organised into shoot primordia. Further growth led to the development of a shoot apex with a distinct tunica corpus and the leaf primordia (Figs. 12, 13). With subsequent subculturing, additional shoots developed with new axillary buds (Fig. 14). They developed into secondary shoot after the third subculture (about 80 to 95 days from first inoculation). These also developed into normal shoots. Another interesting feature was the direct development of free buds on the internodes. They started as small swollen structures attached to the exis at an angle. The transverse section of the axis showed their relationship very clearly and most of them developed from the inner cortical tissue. Leaf primordia were distinct and the ones formed earlier were scale-like with basal meristematic tissue. Obviously they can be used as explants to regenerate further tissue growth (Fig. 15).

Response of leaf tissue on BA media: Young leaves excised from regenerated shoots were inoculated onto media supplemented with BA (0 to 10 ppm). Except for media supplemented with BA (2, 5 ppm) no response was observed in the others. The leaf tissue turned brown on BA (10 ppm) medium in about 4 days after inoculation. Hypertrophic growth was seen in explants at BA medium (2 ppm), resulting in the leaf blade curling up into many folds. On the surface many small outgrowths were also observed.

Anatomical studies showed two growth patterns, (a) the leaf blade was folded upward because of excessive growth in the region of the lower epidermis and, (b) the epidermal and subepidermal layers were wavy, forming many small outgrowths (Fig. 16). At certain points mesophyll cells also divided, contributing to the formation of small outgrowths. Each one of these had a group of actively dividing cells.

Plantlet formation: The excised shoots were planted onto IBA (2.5 and 10 ppm) media to induce rooting and the initial response was slow. At the lower concentration (2 ppm), no root developed, whereas at higher concentrations (5 and 10 ppm), only some of the shoots rooted in about 40 days after inoculation. In each case, only 1 root was produced, growing horizontally to the shoot axis, resembling the primary root of the seedlings (Fig. 6).

Transfer of plantlets into Jiffy-7 pellets: The plantlets with single shoots were transferred to Jiffy-7 pellets which were stored within an enclosed translucent structure. They were each dipped in benlate solution prior to transfer, to avoid fungal infection. The survival rate was about 20%. The leaves were yellowish despite being fed with phostrogen, a soluble fertiliser. The saplings that developed were relatively weak. However, these could be revived with appropriate treatments and grown into healthy plantlets.

Discussion

The possibility of inducing adventitious shoots from callus and nodal segments is described. Thus there is a good potential for adopting *in-vitro* methods to produce propagules in large numbers. However, the average number of shoots that was produced per replicate in the present study was only about 6. Therefore further studies need to be conducted to establish the pattern for production of large number of propagules required for different programmes. Besides using nodal or internodal segments, leaf tissues should be used extensively to induce shoot growth. Large numbers of shoots were induced from leaf tissue of *Fagraea fragrans* Roxb. (Lee & Rao, 1986). In this study, leaf tissue of *S. macrophylla* also responded well in BA media, producing several outgrowths. If each of the outgrowths could be induced to differentiate into shoots, many shoots could be obtained from a single leaf section. Further work is in progress.

For *in-vitro* techniques to be effectively adopted, the plantlets produced must be healthy so as to ensure a high survival rate of quality plants when transferred to soil. This area needs to be refined further since a survival rate of only about 20% of the plantlets was achieved in the present studies. One of the causes could be due to the formation of only a single root which resembled a tap root. More roots might ensure a higher survival rate.

The inability of nodal segments from mature trees to respond is another area of concern. The problem of juvenility factors should be further explored. Various means to induce juvenile shoots to grow from mature plants, either through pruning or grafting, should be attempted in case of elite trees. The newly developed parts should be used as explants.

The present study has re-emphasised that BA is consistently effective in inducing organogenesis in callus or multiple shoot growth in nodal explants of woody plants (Zaerr & Mapes, 1982). Media supplemented with auxin and kinetin combinations were only able to induce callus growth but the presence of BA promoted shoot growth.

Conclusion

With further understanding of the growth responses of various explants and the factors affecting them, there is a good potential for adopting the tissue culture technique for producing propagules of *S. macrophylla* in large numbers. Such studies can be extended to include other useful economically important trees.

Acknowledgement

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